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Review Article

Roles of IL-1α in the Growth of Keratocystic Odontogenic Tumor

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Abstract: Keratocystic odontogenic tumors have a high level of proliferative activity in epithelial cells and they tend to grow aggressively in the jaw. The tumor dramatically decreases in size by decompression of the intracystic fluid pressure. We herein focused on the roles of interleukin (IL)-1α and demonstrated the biochemical mechanisms of the tumor growth. We found that IL-1α is strongly expressed in the lining epithelial cells of the tumors, and the intracystic fluid levels of IL-1α are significantly higher than the levels of the other inflammatory cytokines of IL-6 and tumor necrosis factor-α (TNF-α). The expression of IL-1α in the epithelial cells decreases after the marsupialization of the tumor. *In vitro* experiments also reveal that positive pressure enhances the expression of IL-1α in the tumor epithelial cells in culture. IL-1α stimulates the production of matrix metalloproteinase (MMP)-9, and activates the released proMMP-9 by increasing the expression of proMMP-3 and plasminogen activator urokinase (u-PA) in the tumor epithelial cells. In the fibroblasts isolated from the tumors, IL-1α increases the expression of proMMP-1, proMMP-2, and proMMP-3. IL-1α also activates proMMP-2 by inducing the expression of membrane-type 1 matrix metalloproteinase (MT1-MMP) synergistically with type I collagen. Furthermore, IL-1α increases the expression of macrophage colony-stimulating factor (M-CSF) and cyclooxygenase (COX)-2 in the fibroblasts. The COX-2 synthesizes prostaglandin E_2 (PGE_2), and the secreted PGE_2 stimulates the expression of receptor activator of nuclear factor-κB ligand (RANKL), while neither IL-1α nor PGE_2 affects the expression of osteoprotegerin (OPG) in the fibroblasts. The fibroblasts express Ca^{2+}-sensing receptor (CasR) on the cell surface, and extracellular Ca^{2+} activates COX-2 expression via the CasR. A strong relationship may thus be present between the intracystic fluid pressure and IL-1α expression in epithelial cells, and the released IL-1α may play a crucial role in the growth of keratocystic odontogenic tumors by stimulating proteolytic enzyme production and osteoclastogenesis.

Key words: keratocystic odontogenic tumor, interleukin-1α (IL-1α), matrix metalloproteinase, osteoclastogenesis

I. Introduction

An odontogenic keratocyst is one of the major odontogenic jaw cysts, and it is histologically characterized by a lining of parakeratinized or orthokeratinized stratified squamous epithelium with a fibrous capsule. The cyst has a high potential for proliferative activity of the epithelial cells, and grows more aggressively than the other types of odontogenic jaw cysts with destruction of osteoid extracellular matrices of the bone around the cyst[1]. Recent molecular studies have implicated genetic alterations in odontogenic keratocysts, which supports the clonal/neoplastic nature of the cysts[2]. Odontogenic keratocysts are thus now categorized as odontogenic tumors, and they have been named keratocystic odontogenic tumors according to the WHO classification. Bone

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resorption by odontogenic jaw cysts, including the keratocystic odontogenic tumor, has been thought to be mediated partially by osteoclast-like cells, which are present in the capsule at the tip of the intra-osseous extensions[3], and/or by the biologically active collagenases[4]. However, the precise regulatory mechanisms of the growth of the cysts in the jaw have not been well understood. The inflammatory and multifunctional cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α) have been considered to play a crucial role as osteo-resorptive factors in various bone destructive diseases by stimulating osteoclastogenesis[5–7] and increasing collagenase synthesis[8–10]. In this review, we focused on the inflammatory cytokines in the growth of keratocystic odontogenic tumor, and demonstrated the roles of IL-1α in the growth of the tumor.

II. Inflammatory cytokines in keratocystic odontogenic tumor

IL-1 is mainly expressed in the macrophages, osteoblasts, fibroblasts, muscle cells, endothelial cells, and epithelial cells. IL-1 consists of two agonists IL-1α and IL-1β, which are coded by different genes on chromosome 2q13 and 2q13-21, respectively. They are synthesized as 31 kDa precursor molecules, and are released from the cells after processing to the mature forms, 17 kDa IL-1α and IL-1β. IL-1α and IL-1β can interact with the same receptors, IL-1 receptor type I (IL-1RI) and type II (IL-1RII), although the affinity to each receptor is different. IL-1RI has a long cytoplasmic domain, and acts as a signal transducing receptor, while IL-1RII serves as a decoy receptor for IL-1RI[11]. IL-6 is also expressed in various types of cells such as macrophages, fibroblasts and endothelial cells, and the gene for IL-6 is located on chromosome 7q21-p14. IL-6 is present as glycosylated 21.5 to 28 kDa proteins, and binds to a specific IL-6 receptor (IL-6R) and a soluble form of the IL-6R[12]. TNF-α is a non-glycosylated 17 kDa protein, and its gene is mapped to chromosome 6. TNF-α is synthesized as pro-TNF-α, and activated by TNF-converting enzyme. TNF-α is also expressed in various cells such as macrophages, T lymphocytes, natural killer cells, fibroblasts, smooth muscle cells, and tumor cells[13].

The expression of IL-1α, IL-6 and TNF-α has been demonstrated in the lining epithelium of radicular cysts, dentigerous cysts, and keratocystic odontogenic tumors[14,15]. However, the intensity of the expression of each cytokine is different among the different types of cystic lesions. We have previously demonstrated that in keratocystic odontogenic tumors, the concentration of IL-1α in the fluid is significantly higher than that of IL-1β or TNF-α[15,16]. Furthermore, the concentrations of IL-1α in the fluids of keratocystic odontogenic tumors are significantly higher than those of dentigerous and radicular cysts. Both immunohistochemical and *in situ* hybridization studies have shown the expression of IL-1α mRNA and protein in lining epithelial cells of the tumors to be significantly stronger than that of dentigerous and radicular cysts[15]. Therefore, IL-1α may be one of the key cytokines which regulate keratocystic odontogenic tumor expansion in the jaw. The first question is how the expression of L-1α is regulated in the tumor.

There are two major surgical methods for the treatment of odontogenic jaw cysts, namely marsupialization (Partch I method) and enucleation (Partch II method). Marsupialization is a treatment that creates a surgical window into the cystic cavity. In recent years, marsupialization has been recommended especially for large cystic lesions including keratocystic odontogenic tumor and cystic ameloblastoma in order to avoid making a marked bone defect in the jaw and decreasing the patient's quality of life[17]. The intracystic fluid pressure of the keratocystic odontogenic tumor is significantly higher than atmospheric pressure, and the mean values of the fluid pressure are about 40–50 mmHg[18] (Fig. 1A). Decompression leads to the gradual reduction of bone resorption by the cystic lesions, and reduces the cyst size (Fig. 1B). These clinical findings suggest that intracystic positive pressure might play a crucial role in the regulation of the cyst growth in the jaw. We demonstrated that in the epithelial cells of keratocystic odontogenic tumor tissue specimens, highly expressed IL-1α mRNA and protein were dramatically decreased after marsupialization[15] (Fig. 1C). Furthermore, *in vitro* experiments showed that 60 mmHg of positive pressure increased the IL-1α expression and secretion in the epithelial cells isolated from keratocystic odontogenic tumors[19, 20]. This observation is consistent with the finding that mechanical forces stimulated the secretion of IL-1 in various types of cells such as endothelial cells[21], periodontal ligament cells[22], and keratinocytes[23, 24] (Fig. 1D). Therefore, intracystic positive fluid pressure might

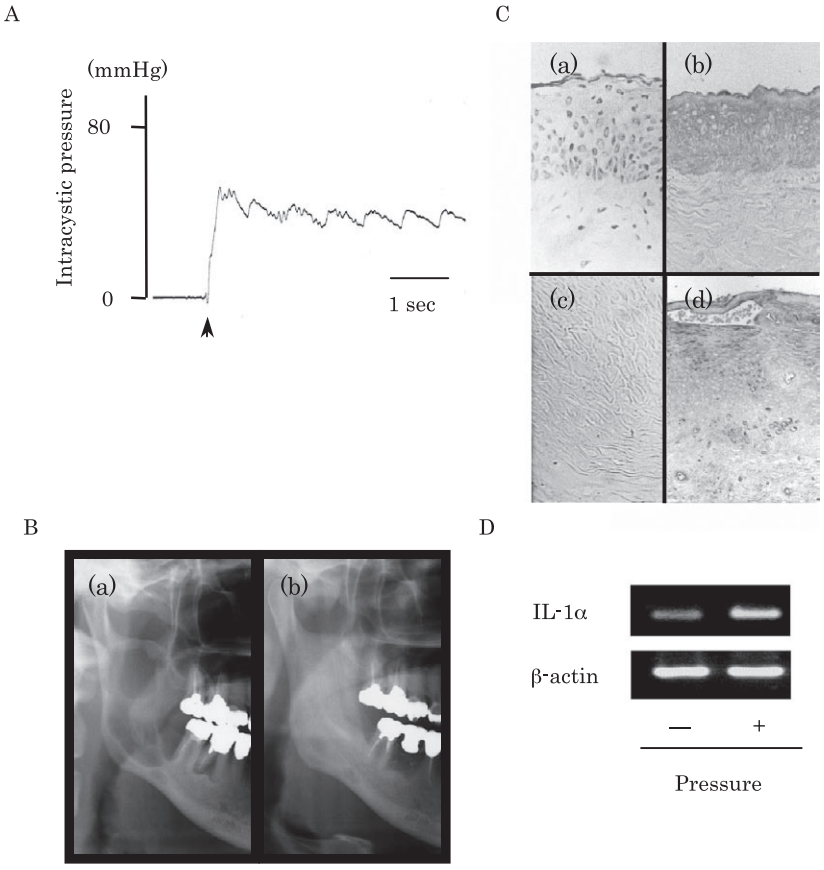

Fig. 1 Intracystic fluid pressure of keratocystic odontogenic tumors.
A: Positive intracystic fluid pressure with oscillation was detected. The horizontal bar indicates 1 second. B: Panoramic radiograms of a case of keratocystic odontogenic tumor before (a) and after (b) marsupialization. After marsupialization, the cyst dramatically decreased in size. C: The expression of IL-1α mRNA (a, c) and protein (b, d) was detected by *in situ* hybridization and immunohistochemistry, respectively before (a, b) and after (c, d) marsupialization in the epithelial cells of keratocystic odontogenic tumor tissue sections. D: The expression of IL-1α mRNA increased by 60 mmHg positive pressure in the epithelial cells isolated from keratocystic odontogenic tumor.

stimulate IL-1α gene expression in the epithelial cells of the keratocystic odontogenic tumor, and play a crucial role in the growth of the tumors in the jaw. However, no significant difference was observed in the fluid pressure among keratocystic odontogenic tumor, dentigerous cyst, and radicular cyst, but the pressure is negatively correlated with the size of the cysts[18]. These findings suggest that the biological characters of the epithelial cells of keratocystic odontogenic tumors might be different from those of other types of odontogenic jaw cysts.

III. Effect of IL-1α on the expression of MMPs

The second question is how IL-1α affects the growth of keratocystic odontogenic tumors in the jaw. Matrix metalloproteinases (MMPs) regulate normal tissue remodeling and pathological processes such as arthritis, tumor metastasis, wound healing, angiogenesis, inflammation, embryonic development, and bone resorption[25]. At present, 25 vertebrate MMPs and 22 human homologues have been identified. Bone extracellular matrices are composed of inorganic and collagenous organic components, and the degradation of the organic matrices is conducted by MMPs and lysosomal cysteine proteinases, such as cathepsin K and L[26]. Several MMPs such as MMP-12, MMP-13, MMP-14, and gelatinases (MMP-2 and MMP-9) have been identified in bone area showing resorption[27, 28]. The gelatinases cleave native collagen types IV, V and X, and also degrade denatured

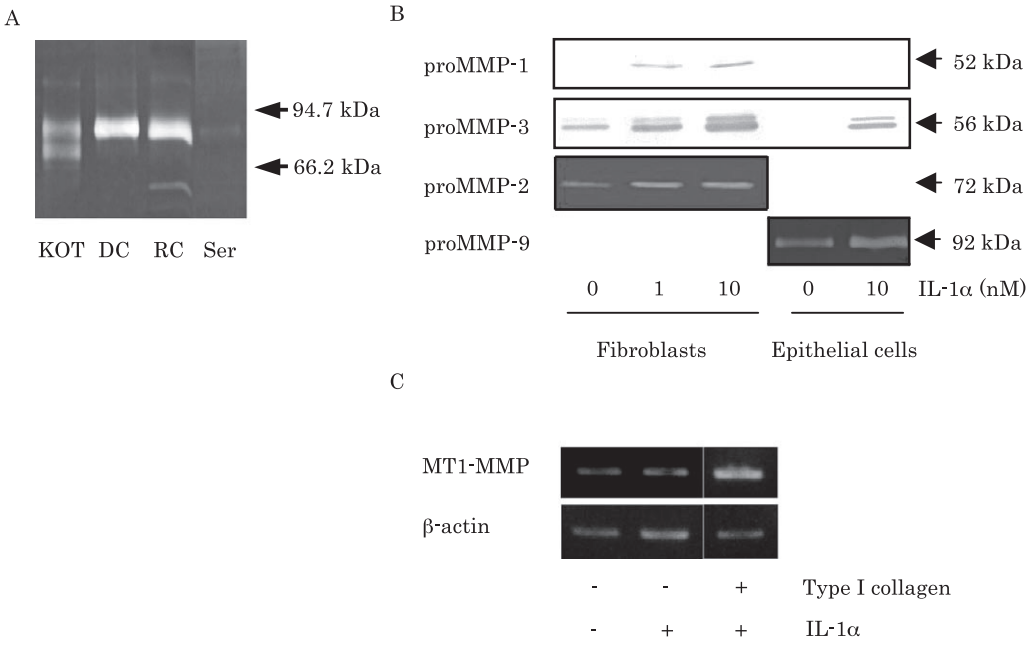

Fig. 2 Expression of MMPs in keratocystic odontogenic tumor.
A: Gelatin zymography of intracystic fluids of keratocystic odontogenic tumor (KOT), dentigerous cyst (DC), radicular cyst (RC), and human serum (Ser). B: The expression of proMMP-1, -2, -3, and -9 in the cells isolated from keratocystic odontogenic tumors. The expression of proMMP-1, -2, and -3 increased after the administration of IL-1α in the fibroblasts isolated from keratocystic odontogenic tumors. The expression of proMMP-9 increased after the administration of IL-1α when the epithelial cells isolated from keratocystic odontogenic tumors were cultured on a fibronectin-coated dish. C: The expression of MT1-MMP and β-actin mRNAs in the fibroblasts isolated from keratocystic odontogenic tumors. The expression of MT1-MMP mRNA increased after the administration of IL-1α in the presence of type I collagen.

collagen such as fibrillar type I, II and III[29]. Since these MMPs are secreted as a latent form (proMMP), the secretion and activation of these MMPs are required prior to working[30, 31]. ProMMP-2 can not be activated by serine proteinases, but can be activated by a unique multiple pathway with membrane type MMPs (MT-MMPs) and tissue inhibitor of metalloproteinase-2 (TIMP-2)[30, 32, 33]. On the other hand, proMMP-9 is activated by tumor cell-derived human trypsin-2 as well as MMP-3, cathepsin G, trypsin and α-chymotrypsin[34, 35]. Previous studies showed the presence of MMP-1, MMP-8, MMP-2 and MMP-9 in homogenized samples of odontogenic jaw cyst walls[4]. We have found that the latent form of MMP-9 is present in the intracystic fluids of keratocystic odontogenic tumors, dentigerous cysts, and radicular cysts, while the active form of MMP-9 is present in the intracystic fluid of keratocystic odontogenic tumors at a higher level than that in dentigerous and radicular cysts (Fig. 2A). Interestingly, neither MMP-2 nor MMP-9 is detected in the fluid of ameloblastomas, and thus we can distinguish these cystic lesions by measuring the gelatinase species in the fluids[16]. IL-1α increases the expression of MMP-9 in the epithelial cells of the keratocystic odontogenic tumors, when the cells are grown on a fibronectin-coated dish[19] (Fig. 2B). Furthermore, IL-1α stimulates the expression of MMP-3 and the plasminogen activator urokinase (u-PA) in the cells[19, 20]. The u-PA activates proMMP-3 in the presence of plasminogen, and the activated MMP-3 activates proMMP-9[19]. In the fibroblasts isolated from the keratocystic odontogenic tumors, IL-1α stimulates the expression of proMMP-1, proMMP-2, and proMMP-3[20] (Fig. 2B). The expression of MT1-MMP is also induced by IL-1α synergistically with type I collagen in the fibroblasts[36] (Fig. 2C). MT1-MMP can degrade a number of extracellular matrix proteins such as gelatin, fibronectin, vitronectin, and type I, II and III collagens, and also can activate proMMP-2. Type I collagen is widely expressed not only in the bone but also in the subepithelial layers of the keratocystic odontogenic tumors.

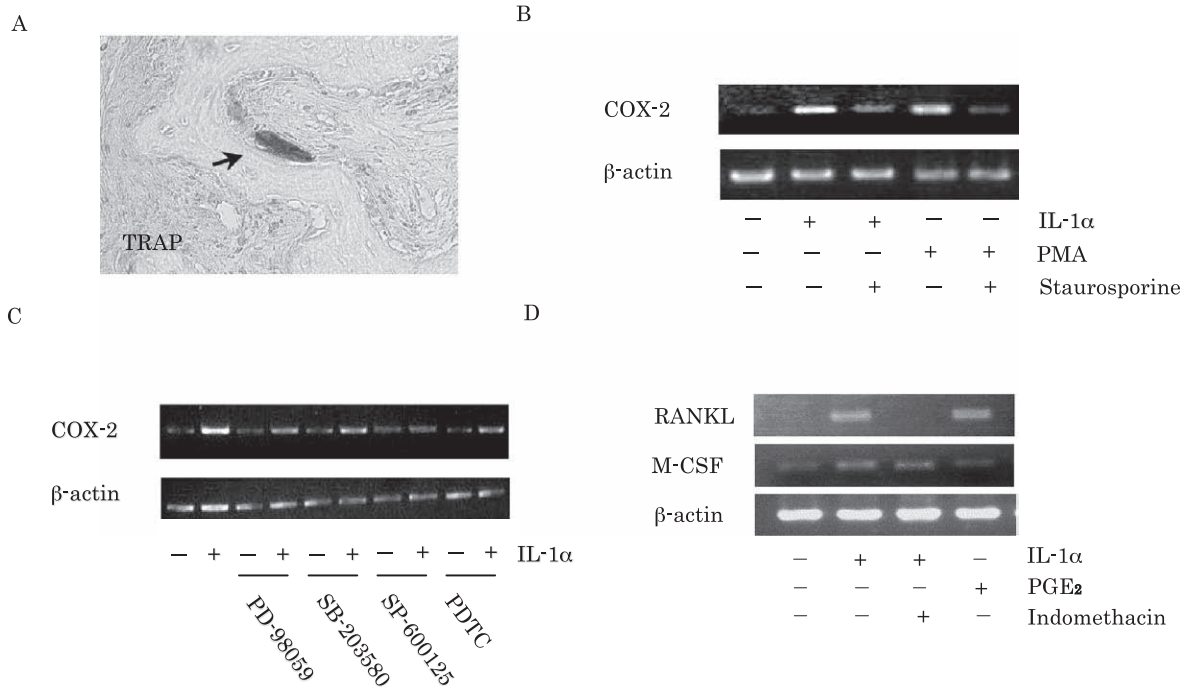

Fig. 3 Effects of IL-1α on osteoclastogenesis in keratocystic odontogenic tumor.
A: TRAP staining in keratocystic odontogenic tumor. The TRAP positive cells were detected at the tip of the intraosseous extensions of the tumor capsule and in direct contact with the bone tissue. B: IL-1α-induced the PKC-dependent expression of COX-2 mRNA in the fibroblasts of keratocystic odontogenic tumor. IL-1α- and PMA-induced expression of COX-2 mRNA was attenuated by staurosporine. C: Effects of inhibitors for ERK1/2 (PD-98059), p38 (SB-203580), JNK (SP-600125), and NF-κB (PDTC) on IL-1α-induced expression of COX-2 mRNA in the fibroblasts isolated from keratocystic odontogenic tumor. D: The expression of RANKL and M-CSF mRNAs in the fibroblasts isolated from keratocystic odontogenic tumor. IL-1α increased the expression of RANKL and M-CSF mRNAs, and the enhancement of RANKL mRNA was attenuated by indomethacin.

Therefore, the expression of IL-1α may induce not only the production of MMP-2, MMP-9, and MT1-MMP but also the activation of these gelatinases, and then stimulate the enzymatic degradation of osteoid extracellular matrices around the keratocystic odontogenic tumors.

VI. Effect of IL-1α on osteoclastogenesis

Osteoclastogenesis is regulated by many factors, such as the receptor activator of nuclear factor κB ligand (RANKL) and its receptor (RANK), the macrophage colony-stimulating factor (M-CSF) and its receptor (c-Fms), and a pseudo-receptor for RANKL, osteoprotegerin (OPG)[37–40]. c-Fms is expressed in osteoclast precursors at the early stage of osteoclastogenesis. M-CSF activates c-Fms, and then enhances RANK expression in the precursors[40]. The binding of RANKL to RANK results in the activation of a TNF receptor-associated factor (TRAF), which activates the NF-κB and JNK pathways in the precursors and eventually stimulates osteoclast differentiation, while OPG inhibits the RANKL-induced osteoclastogenesis. In odontogenic jaw cysts, numerous multinucleated tartrate-resistant acid phosphatase (TRAP)-positive cells were seen at the tip of the intraosseous extensions of the cyst capsule, and were in direct contact with the bone tissue (Fig. 3A). Typical resorption lacunae were identified on the bone surface[3]. These findings suggest that osteoclasts might regulate bone resorption around the cysts, and osteoclastogenesis plays an important role in the cyst growth in the jaw. Prostaglandin E_2 (PGE_2) synthesis requires the conversion of arachidonic acid to prostaglandin H_2 either by cyclooxygenase (COX)-1 or COX-2. COX-1 is expressed constitutively in most cells, while COX-2 is usually undetectable under normal conditions, and its expression is increased by pathological stimulation[41]. The regulation of COX-2 expression is, therefore, pathophysiologically important for PGE_2 synthesis. We showed that COX-2 was expressed in the fibroblasts of keratocys-

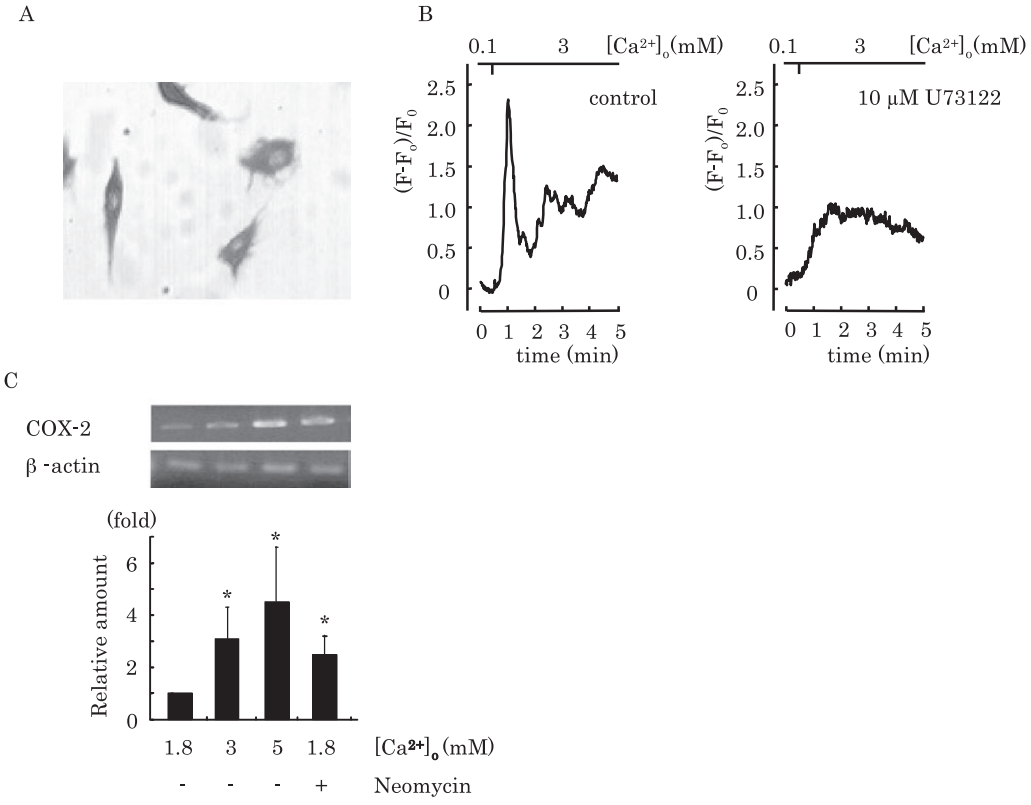

Fig. 4 Effects of extracellular Ca^{2+} in keratocystic odontogenic tumor fibroblasts.
A: Immunocytochemical staining for CasR in the fibroblasts isolated from keratocystic odontogenic tumor. The cells were positively stained with anti-CasR antibody. B: Measurment of the intracellular Ca^{2+} concentration. The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured by fluo-3. After preincubation in 0.1 mM Ca^{2+} HEPES-buffered solution without (left: control) or with phospholipase C inhibitor (right: U-73122), extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) was increased to 3 mM. Phasic increase in $[Ca^{2+}]_i$ was induced by 3 mM $[Ca^{2+}]_o$, and the increase was diminished by U-73122. C: The expression of COX-2 in the fibroblasts isolated from keratocystic odontogenic tumor. Extracellular high Ca^{2+} and CasR activator neomycin stimulated the expression of COX-2 mRNA in the fibroblasts.

tic odontogenic tumor specimens[42]. Furthermore, we have found that IL-1α increases the expression of M-CSF and COX-2 mRNAs in the fibroblasts isolated from keratocystic odontogenic tumors. The IL-1α-induced expression of COX-2 mRNA is regulated both by protein kinase C (PKC)-dependent activation of extracellular signal-regulated protein kinase-1/2 (ERK1/2), p38 mitogen-activated protein kinase (p38 MAPK), and c-Jun N-terminal kinase (JNK) signaling pathways and by NF-κB cascade[42] (Fig. 3B, C). The IL-1α-induced COX-2 activates the production of PGE_2, and then the secreted PGE_2 enhances the expression of RANKL in the fibroblasts[20, 42] (Fig. 3D). RANKL immunoreactivity has been shown to be detected in keratocystic odontogenic tumor specimens, and immunolocalization was also demonstrated to be associated with TRAP-positive cells[43]. Therefore, IL-1α may support osteoclast differentiation by increasing the expression of M-CSF and RANKL in the fibroblasts[20, 42] as in the periodontal ligament cells[42, 44].

The calcium-sensing receptor (CasR) is a seven-transmembrane GTP-binding protein-coupled receptor, which is activated by elevated extracellular Ca^{2+}. The CasR was first cloned from the bovine parathyroid gland[45], and is now known to regulate various pathophysiological functions including thyroid hormone synthesis and secretion[46], calcium reabsorption in the distal tubules in the kidney[47], neurotransmitter release in the nerve[48], and the down-regulation of proliferation and/or differentiation in keratinocytes[49]. We have recently found that CasR is expressed in the fibroblasts isolated from keratocystic odontogenic tumors (Fig. 4A). In the fibroblasts, elevated extracellular Ca^{2+} stimulates the production of inositol(1,4,5)triphosphate and active PKC[50].

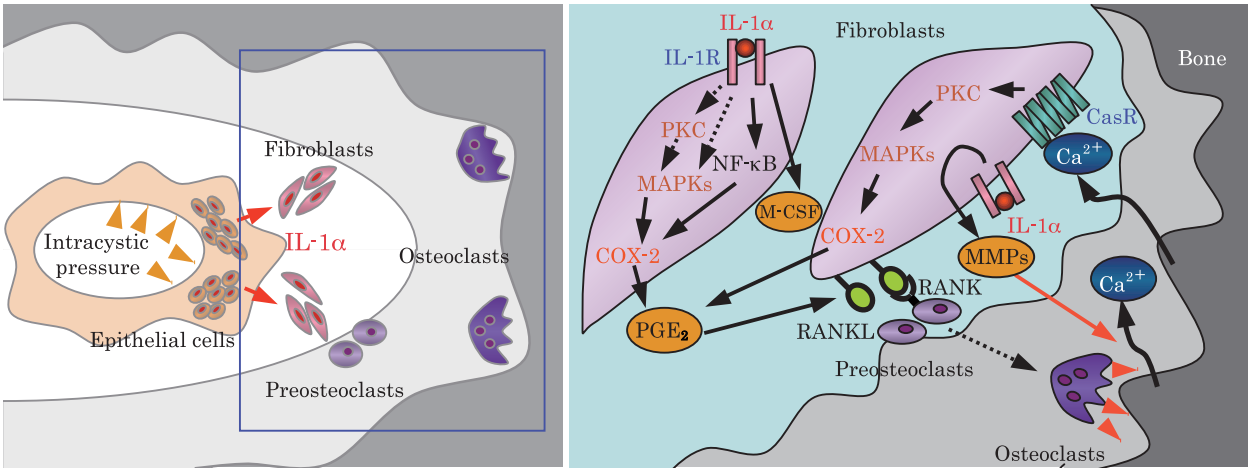

Fig. 5 Role of IL-1α in the growth of keratocystic odontogenic tumors.
Intracystic positive pressure stimulates the expression of IL-1α in the epithelial cells of keratocystic odontogenic tumors (figure on the left). The role of IL-1α in the growth of the tumor is clarified in the right figure. IL-1α increases the expression not only of MMPs, but also of M-CSF and COX-2 in the subepithelial layer fibroblasts. The IL-1α-induced expression of COX-2 is regulated by MAPK and NF-κB signaling pathways. COX-2 synthesizes PGE_2, and the secreted PGE_2 stimulates the expression of RANKL in the fibroblasts. RANKL activates RANK, and stimulates osteoclastogenesis. The released Ca^{2+} from the bone further activates COX-2 expression through CasR expressed on the fibroblasts via MAPK activation. There may thus be a strong relationship among intracystic fluid pressure, IL-1α expression in the epithelial cells, and the stimulation of proteolytic enzyme production and osteoclastogenesis.

As a result, the elevated extracellular Ca^{2+} increases the intracellular Ca^{2+} concentration in the cells and enhances the expression of COX-2 mRNA and protein, and then induces the secretion of PGE_2 by stimulating ERK1/2, p38 MAPK, and JNK through CasR in the fibroblasts[50] (Fig. 4B, C). Therefore, when the bone around the keratocystic odontogenic tumor is absorbed, the released Ca^{2+} from the bone might affect the bone metabolism through the fibroblasts of the tumor.

In summary, IL-1α induced by intracystic positive pressure may therefore play a crucial role in keratocystic odontogenic tumor growth (Fig. 5).

Acknowledgments

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